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## Effects of Saline Water from North Dakota Lakes on Survival of Fathead Minnow (*Pimephales promelas*) Embryos and Sac Fry

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Eggs and sac fry of the fathead minnow (*Pimephales promelas*) were subjected in the laboratory to various dilutions of water obtained from four saline North Dakota lakes; three were sodium sulfate-, one a sodium chloride-type lake. In water from the latter, the percent of fertilized eggs surviving as sac fry for 5 days remained the same as the percent hatched: about 90% at treatment levels of 500–12,000  $\mu$ mho and 68% in undiluted lake water of 18,000  $\mu$ mho. In sodium sulfate-type water, however, about 92% of the fertilized eggs hatched at treatment levels of 500 up to only 6,000  $\mu$ mho, with about 82% living as sac fry for 5 days in water from two of the lakes, and only about 54% living for 5 days in dilutions made from the most saline lake (lake water of 25,000  $\mu$ mho). No sac fry survived for 5 days after hatching in sodium sulfate-type water of 12,000  $\mu$ mho. From laboratory bioassays and field observations, we estimated sodium sulfate-type water exceeding 8,000  $\mu$ mho during reproductive periods may decrease survival of sac fry.

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Les auteurs ont soumis des œufs et des alevins vésiculés de tête-de-boule (*Pimephales promelas*) en laboratoire à diverses dilutions d'eau provenant de quatre lacs salés du Dakota-Nord; trois de ces lacs sont du type sulfate de sodium, l'autre du type chlorure de sodium. Dans l'eau provenant de ce dernier, le pourcentage d'œufs fécondés survivant comme alevin vésiculé pendant 5 jours demeure le même que le pourcentage d'éclosion: environ 90% à des niveaux de traitement de 500–12,000  $\mu$ mho et 68% dans l'eau de lac non diluée de 18,000  $\mu$ mho. Dans l'eau du type sulfate de sodium, cependant, environ 92% des œufs fécondés éclosent à des niveaux de traitement de 500–6,000  $\mu$ mho seulement, et environ 82% survivant comme alevins vésiculés pendant 5 jours dans l'eau des deux lacs; seulement environ 54% survivent pendant 5 jours dans des dilutions obtenues du lac le plus salé (eau de lac de 25,000  $\mu$ mho). Aucun alevin vésiculé ne survécut pendant 5 jours, après incubation dans de l'eau du type sulfate de sodium de 12,000  $\mu$ mho. À la suite d'essais biologiques en laboratoire et d'observations sur le terrain, les auteurs estiment que l'eau du type sulfate de sodium dépassant 8,000  $\mu$ mho pendant la période de reproduction peut diminuer la survie des alevins vésiculés.

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MANY prairie pothole lakes in the upper Great Plains are high in total dissolved solids (TDS)

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in which sulfates often exceed carbonates (Eddy 1966; Northcote and Larkin 1966). Fathead minnows (*Pimephales promelas*) occurring in some of these lakes may be limited in others by high salinities. Fatheads were recorded in one lake in North Dakota with TDS of up to 12,000 mg liter<sup>-1</sup> but died within 40 min when placed in another lake at 23,486 mg liter<sup>-1</sup> (Held and

Peterka 1974). Survival is also partly dependent on ion composition. In Nebraska lakes of the sodium/potassium bicarbonate type, spawning was not successful at TDS greater than 2,000 mg liter<sup>-1</sup> (McCarraher and Thomas 1968), whereas in Saskatchewan lakes of the sodium sulfate types, populations of fatheads were found at TDS of nearly 15,000 mg liter<sup>-1</sup> (Rawson and Moore 1944). The aim of this study was to measure the survival of fathead embryos and sac fry in waters from three sodium sulfate- and one sodium chloride-type lakes.

*Materials and methods* — Minnows collected from North Alkaline and South Lake George, North Dakota were held in aquaria where, under a photoperiod of 14-h daylight, eggs were spawned on the undersides of cement-asbestos pipe half-sections in 25 C dechlorinated city water with a conductivity of 480–815  $\mu\text{mho/cm}$  at 25 C.

Water collected from the following lakes was filtered through 36- $\mu$  nylon screen to remove zooplankters and detritus, and stored at 10–15 C before use: (1) N. Lake George (undiluted water, 22,000–28,000  $\mu\text{mho}$ ), (2) N. Alkaline Lake (undiluted water 12,940–16,300  $\mu\text{mho}$ ), (3) S. Alkaline Lake (undiluted water, 3,640–4,400  $\mu\text{mho}$ ), (4) Kelly's Slough (undiluted water of the sodium chloride type, 18,000  $\mu\text{mho}$ ).

Salinity tolerance tests were run in diluted lake waters with conductivities of 500, 1,300, 4,000, 6,000, 12,000, and 18,000  $\mu\text{mho}$  at water temperatures of  $25 \pm 1$  C in glass test chambers,  $30 \times 30 \times 15$  cm deep. All dilutions were made with distilled water. Oxygen saturation and water circulation were maintained with air stones. A third of the water in each chamber was replaced every 2 days. Twelve hours after fertilization at least 10 eggs were transferred from aquaria to each of four baskets in each test chamber. In each polyvinyl-chloride basket (5 cm diam.  $\times$  9.5 cm deep) a shelf of 316- $\mu$  nylon screen cemented 3 cm below the rim permitted eggs to rest 1 cm below the water surface. One-centimeter holes in the baskets facilitated water circulation as determined by observing movement of methylene blue solution around the baskets. To inhibit fungal growth five drops of aqueous 1% methylene blue were added on days one and two followed by one drop every 2 days. Hatched fry were segregated daily into dated baskets in the same chamber and their survival recorded for 5 days. This was judged long enough to evaluate salinity effects on sac fry without the necessity of beginning feeding. In a trial using city water, all 123 newly hatched fry survived for 7 days without feeding.

Conductivity was determined daily and dissolved oxygen (azide modification of the Winkler method), total alkalinity, and pH every 2 days for each test. Concentrations of calcium and magnesium (atomic absorption), potassium and sodium (atomic emission), sulfate (turbidimetric method, Hach Chemical Company), chloride, and TDS (APHA 1971) were measured for each treatment replicate.

Tests of N. Lake George water were replicated 3 times and most others twice. Salinity effects were assessed by recording percent successful hatch, percent 5-day survival of sac-fry, and percent of sac fry that were normal at hatching. Abnormal fry were determined by the presence of curvature of the spine. Analysis of variance and Duncan's multiple range test were used for statistical assessment.

*Results* — At treatment levels  $\leq 6,000$   $\mu\text{mho}$  about 92% of the fertilized eggs hatched; hatching success declined to about 22% in sodium sulfate water of 12,000  $\mu\text{mho}$  but remained high (98%) in sodium chloride water of 12,000  $\mu\text{mho}$  from Kelly's Slough and was 73% in water of 18,000  $\mu\text{mho}$  (Table 1A). At treatment levels  $\leq 6,000$   $\mu\text{mho}$  about 85% of the fertilized eggs lived as sac fry for 5 days, except in dilutions from N. Lake George where survival was low (about 54%) and erratic (ranging from 5 to 90%) (Table 1B). No sac fry lived for 5 days in water of 12,000  $\mu\text{mho}$  or greater from N. Lake George and N. Alkaline Lake; however, in sodium chloride water from Kelly's Slough survival was 94% in water of 12,000  $\mu\text{mho}$  and 68% in undiluted lake water of 18,000  $\mu\text{mho}$ . A difference in response between sodium sulfate and sodium chloride water of  $\geq 12,000$   $\mu\text{mho}$  was also observed in regard to spinal curvature of hatched fry. In dilutions of water from Kelly's Slough of 12,000  $\mu\text{mho}$  mean percent normal sac fry hatched was 96% but only 21% for N. Lake George and 24% for N. Alkaline Lake (Table 1C). Mean percent normal sac fry in water from N. Lake George of  $\leq 6,000$   $\mu\text{mho}$  was lower (80%) and more erratic (ranging from 50 to 100%) than was recorded in dilutions from other lakes.

In dilutions of sodium sulfate water of similar conductivity of  $\leq 4,000$   $\mu\text{mho}$ , amounts of  $\text{HCO}_3$ ,  $\text{CO}_3$  and Ca tended to be lowest from N. Lake George, higher from N. Alkaline Lake, and highest from S. Alkaline Lake; amounts of Cl,  $\text{SO}_4$ , Na, and Mg were similar for these three lakes (Table 2).

*Discussion* — We are unable to explain the apparent low and erratic survival of sac fry in dilutions of water of  $\leq 6,000$   $\mu\text{mho}$  from N. Lake George, as compared to survival in similar dilutions from N. Alkaline Lake where water chemistry was similar. Perhaps there are insoluble compounds in the very saline water of N. Lake George that were released when the lake water was diluted, thus producing substances toxic to sac fry. Conductivity of undiluted lake water of N. Lake George was about twice that of N. Alkaline Lake.

TABLE 1. Mean percent of A) fertilized eggs hatched, B) fertilized eggs hatching to sac fry that lived for 5 days, and C) sac fry that were normal at hatching, from various dilutions of water taken from four saline lakes, North Dakota. Numbers in parentheses are replications; ranges are given below each mean. About 40 fertilized eggs were used in each experiment.

Lake	$\mu\text{mho}$					
	500	1,300	4,000	6,000	12,000	18,000
A.) N. Lake George	90.9(3)	89.0(3)	94.8(3)	93.2(3)	30.8*(3)	
	85.1–95.0	70.0–97.5	74.0–100	84.0–97.5	20.0–31.8	
N. Alkaline	96.7(1)	97.5(2)	93.8(2)	92.5(1)	12.5*(2) <sup>a</sup>	
		97.5–97.5	90.0–97.5		12.5–12.5	
S. Alkaline	83.7(2)	91.3(2)	87.5(2) <sup>b</sup>			
	80.0–87.5	90.0–92.5	77.5–97.5			
Kelly's Slough	86.7(2)	87.5(2)	97.5(2)	93.8(2)	97.5(2)	72.5(1)
	73.3–100	75.0–100	97.5–97.5	87.5–100	97.4–97.5	
B.) N. Lake George	61.1(3)	50.8(3)	50.4(3)	54.4(3)	0.0*(3)	
	29.8–90.0	5.0–87.5	11.1–85.0	22.7–80.5	0.0–0.0	
N. Alkaline	90.0(1)	87.8(2)	72.5(2)	70.0(1)	0.0*(2) <sup>a</sup>	
		80.9–95.0	62.5–82.5		0.0–0.0	
S. Alkaline	82.5(2)	80.0(2)	93.9(2) <sup>b</sup>			
	70.0–95.0	70.0–90.0	90.–3–97.4			
Kelly's Slough	85.0(2)	87.5(2)	95.0(2)	91.4(2)	93.5(2)	67.5(1)
	70.0–100	75.0–100	92.5–97.6	85.0–97.8	89.5–97.5	
C.) N. Lake George	72.9(3)	83.3(3)	79.8(3)	82.3(3)	20.6*(3)	
	50.0–89.7	50.0–100	53.7–100	59.7–100	8.8–40.8	
N. Alkaline	93.1(1)	100(2)	94.9(2)	100(1)	24.0*(2) <sup>a</sup>	
		100–100	89.7–100		10.5–37.5	
S. Alkaline	85.4(2)	87.8(2)	95.5(2) <sup>b</sup>			
	81.3–89.5	78.4–97.3	93.6–97.4			
Kelly's Slough	97.8(2)	100(2)	97.4(2)	98.9(2)	96.0(2)	93.1(1)
	95.7–100	100–100	94.9–100	97.8–100	91.9–100	

<sup>a</sup>Undiluted lake water of 12,940–16,000  $\mu\text{mho}$  was used.

<sup>b</sup>Undiluted lake water of 3,640–4,400  $\mu\text{mho}$  was used.

\*Statistically significant ( $P \leq .05$ ) when compared to other means in the row as determined by Duncan's multiple range test using numerical data.

Slight decreases in dissolved oxygen with increased salinity apparently had little influence on sac fry survival as survival was good in water from Kelly's Slough where dissolved oxygen decreased by the same magnitude as in sodium sulfate-type water of similar salinity.

Although antagonistic and synergistic ion effects were not assessed, general observations were that death of sac fry in water of 12,000  $\mu\text{mho}$  of the sodium sulfate type was associated with: 1) sulfates exceeding 8,000 mg liter<sup>-1</sup>; tolerance limits of fatheads were 7,500 mg liter<sup>-1</sup> in sodium/magnesium sulfate lakes in Saskatchewan (Rawson and Moore 1944), 2) total alkalinity exceeding 1,000 mg liter<sup>-1</sup>; more than 2,000 mg liter<sup>-1</sup> caused a sharp decline in spawning success of fatheads in Nebraska saline lakes (McCarraher and Thomas 1968), 3) potassium exceeding 300 mg liter<sup>-1</sup>; potassium averaged 236 mg liter<sup>-1</sup> (extreme of 500 mg liter<sup>-1</sup> in one lake) in 16 Nebraska lakes where fatheads survived for 6 or more months (McCarraher and

Thomas 1968), 4) calcium levels of 7–10 mg liter<sup>-1</sup>; low calcium may increase toxicity of other chemical compounds (McKee and Wolf 1963). High pH's of 9.2–9.3 were probably not at lethal limits as pH's as high as 9.4 (mean of 9.2) were observed in N. Alkaline Lake during ice-free periods 1969–70 (Held 1971). pH's near 9.8 were tolerated by fathead minnows in Nebraska saline lakes (McCarraher 1962). Conductivity appears to be an appropriate expression of salinity for the various treatments rather than TDS. For example, in water of 12,000  $\mu\text{mho}$ , millimoles per liter (osmotic concentration) were nearly equal for sodium sulfate and sodium chloride water (288 and 266, respectively), as compared to TDS of 12,098 mg liter<sup>-1</sup> and 8,761 mg liter<sup>-1</sup>, respectively.

Laboratory indications that fry survive at 6,000, but not at 12,000  $\mu\text{mho}$  of the sodium sulfate-type water, were reinforced by field observations that waters exceeding 7,000–8,000  $\mu\text{mho}$  may prevent successful reproduction. We

TABLE 2. Mean physical and chemical characteristics of various lake water solutions ( $\mu\text{mho}/\text{cm}$  at 25 C) for North Lake George (N.G.), North Alkaline (N.A.), South Alkaline (S.A.) and Kelly's Slough (K.S.), North Dakota. All chemical measurements are in milligrams per liter<sup>-1</sup>. Means were based on three separate measurements in N.G.; two in the other lakes.

$\mu\text{mho}$	Lake	Total dissolved solids	pH	O <sub>2</sub>	HCO <sub>3</sub>	CO <sub>3</sub>	Cl	SO <sub>4</sub>	Ca	Mg	K	Na
500	N.G.	256	7.6	8.0	29	0	16	156	<1	8	9	76
	N.A.	268	6.7	7.6	51	0	27	160	<1	9	14	76
	S.A.	271	7.6	7.8	79	0	13	137	2	12	13	69
	K.S.	248	6.6	7.8	6	0	101	57	10	6	4	64
1,300	N.G.	788	8.3	8.0	72	1	45	415	1	25	16	221
	N.A.	764	8.1	7.6	149	2	44	463	1	24	31	229
	S.A.	748	8.6	7.7	192	17	31	355	3	32	26	196
	K.S.	690	6.8	7.7	11	0	285	115	25	17	7	187
4,000	N.G.	2,614	8.8	7.6	198	48	183	1,480	3	96	84	831
	N.A.	2,557	8.9	7.4	366	88	128	1,430	3	78	104	593
	S.A.	2,621	9.0	7.5	588	175	105	1,320	6	112	116	636
	K.S.	1,868	7.3	7.7	25	0	838	365	70	47	29	489
6,000	N.G.	4,869	9.0	7.4	318	102	300	3,258	4	157	146	1,333
	N.A.	4,469	9.1	7.2	566	216	230	2,900	5	135	176	996
	K.S.	3,679	7.4	7.6	33	0	1,606	810	122	96	52	943
12,000	N.G.	12,098	9.2	7.1	565	360	733	7,800	7	423	318	3,338
	N.A. <sup>a</sup>	13,639	9.3	6.4	1,950	902	688	8,800	10	397	537	4,075
	K.S.	8,761	7.8	7.3	66	0	3,775	2,225	275	244	120	2,640

<sup>a</sup>Undiluted lake water (mean of 14,600  $\mu\text{mho}$ ).

observed on July 14, 1973 that fathead minnow eggs spawned on floating boards placed near the shore of N. Alkaline Lake, where lake water conductivity was 8,000  $\mu\text{mho}$ , had lost their adhesiveness and were easily lost from the boards; all eggs removed to the laboratory died. We observed that adult fatheads survived in N. Alkaline Lake when winter conductivities were 16,000  $\mu\text{mho}$  with successful reproduction in the spring and early summer when conductivities ranged from 6,000 to 7,000  $\mu\text{mho}$ .

Although laboratory experiments demonstrated that 68% of fertilized eggs hatched into sac fry in undiluted water of 18,000  $\mu\text{mho}$  from Kelly's Slough, we found no fatheads in the lake, but did collect the brook stickleback (*Culaea inconstans*) and banded killifish (*Fundulus diaphanus*). We did not determine why fathead minnows were not found.

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